DETECTION OF PIA2 GENE POLYMORPHISM IN GLYCOPROTEIN IIIa IN PATIENTS WITH MIGRAINE

by

DR. SHALINI BHASKAR
M.B., B.S.

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE)

UNIVERSITI SAINS MALAYSIA
MAY 2006

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Glycoprotein IIIa (GP IIIa) is a platelet membrane receptor, which when activated leads to platelet adhesion. Platelet alloantigen (P1A) is normally represented on the GPIIIa of human platelet membrane in the more common homozygous allelic state (P1A1/A1), or the rarer polymorphic state (P1A1/A2). The later polymorphism P1A1/A2 polymorphic state renders the platelet hyperadhesive leading to increased incidence of coronary events and possibly migraine as well. Migraine is also a disorder wherein platelet hyperadhesion and serotonin release have been observed. Migraine is a common headache disorder exhibiting a prevalence of 9% in the Malaysian population.

This study was designed to identify the prevalence of the homozygous (P1A1/A1) and the polymorphic (P1A1/A2) state in the population at Kelantan and to determine whether the polymorphic state (P1A1/A2) is more common in our migraine patients. So far no work has been published from Malaysia on this subject.

A case control study was conducted between September 2004 and October 2005. eighty (80) patients who fulfilled the International Headache Society (IHS) criteria for migraine with or without aura, and a group of eighty healthy volunteers were recruited for the study. The P1A1/A2 genotype pattern of all these 160 individuals was analysed by polymerase chain reaction (PCR) using the Allele Specific Oligonucleotide (ASO) technique and the results compared with the nigraine symptoms in the patients concerned.

It was found that 77 (of the 80) controls and 76 (of the 80)cases with migraine possess the homozygous (P1A1/P1A1) configuration, indicating that in the population here the homozygous state is more common (i.e present in 153 out of 160 individulas studied). Secondly, the occurrence of the P1A1/A2 polymorphism in only four (of the 80) migraine cases and also three (of the 80) controls suggest that the polymorphic (P1A1/A2) state is not more frequent in the migraine cases. Thirdly, of the four cases positive for P1A1/A2 polymorphism three had classical visual aura (75%).

Earlier studies have reported that migraine with aura has an increased familial incidence when compared with migraine without aura suggesting that migraine with aura could well be related to the inheritance of this P1A1/A2 polymorphism state.

Although our findings do not totally support the hypothesis that the P1A2 polymorphism represents an added inherited platet risk factor for migraine or even migraine with aura, further searches for such a factor are clearly warrwnted, because of the familial aggregation of migraine headache cases.

Thus this preliminary study shows that P1A1/A2 polymorphic state on the GPIIIa platelet membrane receptor does not increase the risk of inheriting migraine. However if present, it is more likely to manifest as migraine with aura in the migraineurs with this polymorphism.

4) Sila sediakan Laporan teknikal lengkap yang menerangkan keseluruhan projek ini. [Sila gunakan kertas berasingan]
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List a glosssary that explains or reflects your research:

Bahasa Malaysia	Bahasa Inggeris
Gen P1A2	P1A2 gene
.....	.....
Glycoprotein IIIa	Glycoprotein IIIa
.....	.....
Migrain	Migraine
.....	.....

5) **Output Dan Faedah Projek**
Output and Benefits of Project

(a) * Penerbitan *(termasuk laporan/kertas seminar)*
Publications (including reports/seminar papers)
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1. Detection of human platlet alloantigens (HPA 1a/1b)/P1A1/A2 polymorphism on the platelet glycoprotein IIIa (GPIIIa) in migraine patients. 2nd Asian Symposium on tRansfusion Medicine and Alternatives on 14-15th January 2005.
2. Detection of P1A2 gene polymorphism in glycoprotein IIIA in patients with Migraine. Journal of Neurology 13 (Suppl 2): pg 152.

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten atau impak kepada dasar dan masyakarat.**
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The benefits of any scientific study is gauged according to the usefulness of the results and observations when implemented practically to the community- for example, the discovery of a new drug, procedure, cost-effective treatment, diagnostic methodology etc. This was mainly a study to verify the hypothesis of the P1A1/P1A2 polymorphism and its influence on migraine, based on the platelet behaviour. However, certain observations that could prove beneficial are : understanding the link between the genetics and the clinical presentation of the migraine syndrome and development of newer drug for treatment of migraine (especially those with aura) based on the presence or absence of P1A2 polymorphism. In any case, this study has been the first of its kind to bring to light the P1A1/P1A2 status in Kelantan population.

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Dr Shalini Bhaskar , MMed (Internal Medicine) and will be graduated on August 2007.

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Equipment that has been purchased:

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KOMEN JAWATANKUASA PENYELIDIKAN PUSAT PENGAJIAN
Comments of the Research Committees of Schools/Centres

All 3 objectives of the study were achieved; study completed. one M.Med dissertation produced.

PROF. MADYA MUSTAFFA MUSA
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[Research Committee of School/Centre]

23/5/07
TARIKH
Date

Updated : 16MAC2006

BORANG LAPORAN HASIL PENYELIDIKAN

PPSP

Tajuk geran: Detection of P1A2 gene polymorphism in glycoprotein IIIa in patients with migraine.

Penyelidik: Prof. Dr Jafri Malin Abdullah
Jenis geran: USM Short Term
Tempoh geran: September 2004 hingga Ogos 2006

Jenis laporan: Laporan Kemajuan ☐

Laporan Akhir*: ☑

OBJEKTIF SPESIFIK KAJIAN (sama spt dalam proposal)	SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL	OBJEKTIF TERCAPAI ATAU TIDAK
1. To determine the frequency of P1A1 and P1A2 polymorphism in migraine patients and normal volunteers in Kelantan.	Status polimorphisma P1A1/A2 bagi GPIIIa reseptor membran dapat dikenalpasti frekuensinya dalam pesakit migraine.	Tercapai.
2. To determine any association between the presence of P1A2 polymorphism and the occurrence of migraine headache.	Polimorphisma P1A2 menyebabkan peningkatan risiko platelet kepada pesakit migraine dengan aura.	Tercapai.
3. To investigate the clinical features of migraine in patients with P1A polymorphism.	Mengikut kajian yang dijalankan, penyakit migraine ini juga melibatkan genetik.	Tercapai.
4.		

** Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan. Senarai peralatan yang dibeli di bawah geran juga perlu diserahkan.*

Nama Penyelidik Utama (PI): Prof.Dr Jafri Malin Abdullah
Tarikh: 12 Mac 2007

Professor Dr. Jafri Malin Datuk Hj. Abdullah
Professor of Neurosciences and Senior Consultant Neurosurgeon
AM (Mal), MD (USM), FADUSM, Diplomate Neurosurgery (Belgium), PhD (Belgium), FRCS (USA), FACS (USA) FWFNS (Switzerland), FRCS (Edinburgh).
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Gazetted Neurosurgeon Government of Malaysia (46) KKM 87 P131/201/1 NO 5819
Head Department of Neurosciences
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16150 Kubang Kerian, Kelantan, Malaysia.

+738T/C polymorphism in exon 4 of NFKBIL1 gene results in an amino acid substitution (C224R) which may be of functional relevance, as cysteine is a highly reactive and structurally significant amino acid. Results of a German study suggested that +738C allele of the T/C NFKBIL1 polymorphism was a risk factor for MS, especially among patients with relapsing / remitting course of MS. The aim of the present study was to investigate whether NFKBIL1 T/C polymorphism is associated with MS in a Polish population.

Methods: We analyzed 107 MS patients and 110 unrelated healthy controls matched for age and sex. +738T/C polymorphism of the NFKBIL1 gene was investigated using the single strand conformation polymorphism method.

Results: The distribution of +738T/C alleles of NFKBIL1 gene was similar among MS patients (9.4%) and controls (8.2%; p=NS). There was no correlation between polymorphism distribution and clinical course of the disease.

Conclusions: We failed to find an association between +738T/C polymorphism of NFKBIL1 gene and MS in a Polish population.

P1425

DETECTION OF P1A2 GENE POLYMORPHISM IN GLYCOPROTEIN IIIA IN PATIENTS WITH MIGRAINE

B. Shalini[1], A. Prasad[1], N. Mohd Yusoff[2], A.M. Hussin[1], W.Z. Abdullah[1], J. Abdullah[3]

[1]Department of Medicine, [2]Human Genome Center, [3]Department of Neurosciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Glycoprotein IIIa (GP IIIa) is a platelet membrane receptor, which when activated leads to platelet adhesion. Platelet alloantigen (P1A) is normally represented on the GPIIIa of human platelet membrane in the more common homozygous allelic state (P1A1/A1), or the rarer polymorphic state (P1A1/A2). Migraine is also a disorder wherein platelet hyperadhesion and serotonin release have been observed. Migraine is a common headache disorder exhibiting a prevalence of 9% in the Malaysian population. This study was designed to identify the prevalence of the homozygous (P1A1/A1) and the polymorphic (P1A1/A2) state in the population at Kelantan and to determine whether the polymorphic state (P1A1/A2) is more common in our migraine patients. A case control study was conducted between September 2004 and October 2005. 80 patients who fulfilled the International Headache Society (IHS) criteria for migraine with or without aura, and a group of 80 healthy volunteers were recruited. The P1A1/A2 genotype pattern of all these 160 individuals was analysed by polymerase chain reaction (PCR) using the Allele Specific Oligonucleotide (ASO) technique and the results compared with the migraine symptoms in the patients concerned. It was found that 77 controls and 76 cases with migraine possess the homozygous (P1A1/P1A1) configuration, indicating that in the population here the homozygous state is more common. Secondly, the occurrence of the P1A1/A2 polymorphism in only four migraine cases and three controls suggests that the polymorphic (P1A1/A2) state is not more frequent in the migraine cases. Thirdly, of the four cases positive for P1A1/A2 polymorphism three had classical visual aura (75%). Thus this preliminary study shows that P1A1/A2 polymorphic state on the GPIIIa platelet membrane receptor does not increase the risk of inheriting migraine.

P1426

CUMULATIVE EFFECT OF E2 OR E4 ALLELE OF APO E GENE AND HYPERTENSION AS A RISK FACTOR OF DEEP BRAIN HEMORRHAGE

P. Szczudlik[1], A. Slowik[2], D. Wloch[2], J. Pera[2], T. Dziedzic[2], W. Turaj[2], A. Szczudlik[2]

[1]Department of Neurology, Warsaw Medical Academy, [2]Department of Neurology, Jagiellonian University Krakow, Warsaw, Poland

Studies suggest that APO E polymorphism plays a role as a risk factor of brain haemorrhage. We studied the significance of APO E common polymorphism in patients with deep brain haemorrhage (DBH) as compared to healthy controls. We genotyped 119 patients with DBH and 228 controls matched for age and sex. Stroke patients underwent computed tomography, angiography, and where needed MRI/MRA to exclude vascular malformations, haemorrhage to tumour amyloid angiopathy, etc. Univariate analysis showed that genotypes with E2 or E4 allele (genotypes at risk) were significantly over represented in the cases [E2E3 – 19 (16%); E2/E4-1 (0.8%), E3/E3 – 72 (60.5%), E3/E4 – 27 (22.7%) E4/E4-0 (0%)] when compared with controls: E2/E2 – 3 (1.3%) E2E3 – 19 (9.6%); E2/E4-4 (1.8%), E3/E3 – 165 (72.4%), E3/E4 – 37 (16.3%), $p<0.05$]. A logistic regression analysis adjusted for age, sex and hypertension revealed that the genotypes at risk were not longer independent risk factors of DBH (OR=1.53, 95% CI:0.92–2.56). We studied the interaction between hypertension and genotypes at risk in patients with DBH. Adjusted OR of stroke for normotensive subjects carrying genotypes at risk when compared with normotensive carriers of E3E3 genotype was 1.91 (95% CI:0.57–6.41). Adjusted OR of stroke for hypertensive subjects carrying E3E3 genotype when compared with normotensive carriers of E3E3 genotype was 8.49 (95% CI:3.75–19.2). An increased risk of stroke was found in hypertensive subjects carrying genotype at risk when compared with normotensive carriers of E3E3 genotype. Adjusted OR was 13.2 (95% CI: 5.50–31.8). The presence of APO-E2 or E4 allele in hypertensive subjects increases the risk of stroke by 1.5 when compared with hypertensive carries of E3E3 genotype. Genotypes at risk in normotensive subjects are not risk factors for DBH.

P1427

MYOTONIC DYSTROPHY TYPE 2

S. Vohanka[1], J. Bednarik[1], L. Fajkusova[2], J. Sedlackova[2]

[1]Department of Neurology, [2]Department of Molecular Biology and Gene Therapy, IHOK, University Hospital, Brno, Czech Republic

Background and aims: Myotonic dystrophy type 2 (MD2) is a dominantly inherited disorder with multisystemic clinical features. The disease is caused by a CCTG repeat expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene. If compared with myotonic dystrophy type 1 (MD1), it is a variant occurring mainly in Europe and considered of lesser frequency. Both types share a number of common clinical features, but also some dissimilarity in the clinical manifestation. MD2 was not described in the Czech population to this time.

Methods: A PCR analysis and repeat-primed PCR were carried out in a population of patients with clinical and/or electrophysiological signs of myotonic myopathy in whom no mutation corresponding with MD1 was found.

Results: During 2004–2005 30 patients with positive mutations in ZNF9 were discovered: 7 cases were sporadic, 23 patients

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 JANUARI 2007

Jumlah Geran:	RM	Tiada Rekod	Ketua Projek:	DR. ATUL PRASAD/ PROF JAFRI MALIN ABDULLAH
Peruntukan 2004 (Tahun 1)	RM	10,000.00	Tajuk Projek:	Detection of PIA2 Gene Polymorphism of Platelet Glycoprotein(GPIIIa) in Patients with Migraine Headache
Peruntukan 2005 (Tahun 2)	RM	10,000.00		
Peruntukan 2006 (Tahun 3)	RM	0.00	Tempoh:	1 Sept 04-31 Ogos 06
			No.Akaun:	304/PPSP/6131347

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
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304	22000	PPSP	6131347		-	-	-	-	-	-	-
304	23000	PPSP	6131347		300.00	-	300.00	-	-	-	300.00
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304	25000	PPSP	6131347		-	-	-	-	-	-	-
304	26000	PPSP	6131347		-	-	-	-	-	-	-
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304	32000	PPSP	6131347		-	-	-	-	-	-	-
304	35000	PPSP	6131347		-	-	-	-	-	-	-
					20,000.00	11,136.00	8,864.00	7,588.40	-	8,226.50	1,275.60

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MAY 2006

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This dissertation would not have been possible without the continuous help and support from many people, especially my professors, fellow colleagues and my family.

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My sincere gratitude and credit to my supervisor Dr. Narazah Mohd Yusoff for her enduring support and encouragement. If not for her guidance and endurance this study would not be complete.

I would like to take this opportunity to thank my supervisor and senior mentor Professor Dr. Jafri Malin Abdullah, who has given me all the support and guidance in completing this dissertation.

I would especially like to thank my supervisors Dr.Wan Zaidah Abdullah and Dr. Ahmad Munawwir Husin, for their assistance through out the research project. Special thanks to Associate Professor Dr. Syed Hatim and Dr. Zaharah Sulaiman for their support and co-operation in the statistical analyses.

Appreciations also go to Miss Surini Yusoff and the senior laboratory technologist Miss Selamah Ghazali who have helped me immensely with the laboratory work.

Last but not least, I would like to convey my deepest thanks to my respected husband, Dr. Ananda Dorai and my lovely children, who have given me the support and co-operation right from the start. I am grateful and thankful for all the sacrifices and hardships that we went through to help me complete this dissertation. Finally, I would like to dedicate this dissertation to my beloved parents for their constant support and sacrifices.

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ABBREVIATIONS

GP – Glycoprotein

PlA1/A2 – Platelet alloantigen A1/A2

CSD - Cortical Spreading Depression

CGRP - Calcitonin gene-related peptide

PET - Positron emission topography

5-HT - 5-hydroxytryptamine

CHD - Coronary heart disease

vWF - von Willebrand factor

HPA- Human Platelet Alloantigen

NAIT - Neonatal alloimmune thrombocytopenia

PCR - Polymerase Chain Reaction

DNA – Deoxyribonucleic acid

A- Adenine

T- Thymidine

G – Guanine

C- Cytosine

ASO - Allele specific oligonucleotide

RFLP - Restriction fragment length polymorphism

HUSM – Hospital Universiti Sains Malaysia

MIDAS – Migraine disability assessment score

EDTA - Ethylenediamine tetraacetic acid

HGH – Human Growth Hormone

ABSTRAK

Glikoprotein IIIa ialah membran platelet yang bila diaktifkan, menyebabkan adhesi platelet. Alloantigen platelet (PlA) pada kebiasaannya berada pada GPIIIa membrane platelet dalam keadaan yang homozigus alel (PlA1/A1) ataupun sebahagian kecil berada dalam status polimorphisma (PlA1/A2). Status polimorphisma (PlA1/A2) menyebabkan platelet menjadi terlebih adhesive menyebabkan peningkatan penyakit koronari dan juga kemungkinan dalam migrin. Migrin adalah penyakit dimana adhesi platelet meningkat dan rembesan serotonin berlaku.

Migrin adalah sejenis sakit kepala yang lazim berlaku dengan prevalen sebanyak 9% dalam populasi Malaysia.

Kajian ini dirangka untuk mengenalpasti prevalen homozygis (PlA1/A1) dan status polimorphisma (PlA1/A2) dikalangan penduduk di Kelantan dan untuk mengenalpasti sama ada polimorphisma (PlA1/A2) adalah lebih kerap dalam penyakit migrin. Setakat ini tiada kajian sebegini yang telah diterbitkan di Malaysia dalam subjek ini.

Satu kajian kes control telah dijalankan dari September 2004 dan Oktober 2005. Lapan puluh pesakit yang memenuhi criteria persatuan sakit kepala internasional (IHS) bagi migrin dengan aura dan migrin tanpa aura, disamping itu juga sekumpulan lapan puluh orang sukarelawan telah masukkan didalam kajian ini.

Corak genetik PlA1/A2 bagi kesemua 160 orang individu telah dianalysi menggunakan teknik "PCR" dan "Allele Specific Oligonucleotide (ASO)" dan keputusannya dibandingkan dengan simptom pesakit terlibat.

Adalah dijumpai bahawa 77 (dari 80) control dan 76 (dari 80) kes migrin mempunyai konfigurasi homozigus (PlA1/A1), menunjukkan dalam populasi ini status homozigus adalah lebih kerap (dalam 153 daripada 160 individu yang dikaji).

Keduanya, polimorphisma PlA1/A2 berlaku dalam 4 kes (dari 80) migrin dan 3 (dari 80) control menunjukkan status polimorphisma (PlA1/A2) tidak berlaku dengan lebih kerap dalam kes migrin.

Ketiganya, bagi keempat – empat kes yang positif untuk PlA1/A2 menpunyai simptom aura visual (75%). Kajian terdahulu mendapati bahawa pesakit migrin dengan aura insidennya meningkat di kalangkan keluarga bila banding dengan migrin tanpa aura menunjukkan migrin dengan aura mungkin berkait dengan status polimorphisma PlA1/A2.

Walaupun keputusan kajian ini tidak menyokong sepenuhnya hipotesis dimana polymorphisma PlA2 menyebabkan peningkatan risiko platelet kepada pesakit migrin dengan aura. Penyelidikan lanjut adalah diperlukan, kerana terdapat pesakit keluarga bagi sakit kepala jenis migrin.

Jadi kajian ini mendapati status polimorphisma PlA1/A2 bagi GPIIIa reseptor membrane platelet telah meningkatkan risiko menghadapi migrin, walaubagaimanapun jika ada lanya adalah lebih berkaitan dengan migrin dengan aura dikalangan pesakit migrin dengan polymorphisma ini.

ABSTRACT

Glycoprotein IIIa (GP IIIa) is a platelet membrane receptor, which when activated leads to platelet adhesion. Platelet alloantigen (PlA) is normally represented on the GPIIIa of human platelet membrane in the more common homozygous allelic state (PlA1/A1), or the rarer polymorphic state (PlA1/A2). The latter polymorphic PlA1/A2 polymorphic state renders the platelet hyperadhesive leading to increased incidence of coronary events and possibly migraine as well. Migraine is also a disorder wherein platelet hyperadhesion and serotonin release have been observed. Migraine is a common headache disorder exhibiting a prevalence of 9% in the Malaysian population.

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A case control study was conducted between September 2004 and October 2005. Eighty (80) patients who fulfilled the International Headache Society (IHS) criteria for migraine with or without aura, and a group of eighty healthy volunteers were recruited for the study. The PlA1/A2 genotype pattern of all these 160 individuals was analysed by polymerase chain reaction (PCR) using the Allele Specific Oligonucleotide (ASO) technique and the results compared with the migraine symptoms in the patients concerned.

It was found that 77 (of the 80) controls and 76 (of the 80) cases with migraine possess the homozygous (PlA1/PlA1) configuration, indicating that in the population here the homozygous state is more common (i.e present in 153 out of 160 indiviuals studied). Secondly, the occurrence of the PlA1/A2 polymorphism in only four (of the 80) migraine cases and also three (of the 80) controls suggest that the polymorphic (PlA1/A2) state is not more frequent in the migraine cases. Thirdly, of the four cases positive for PlA1/A2 polymorphism three had classical visual aura (75%).

Earlier studies have reported that migraine with aura has an increased familial incidence when compared with migraine without aura suggesting that migraine with aura could well be related to the inheritance of this PlA1/A2 polymorphic state.

Although our findings do not totally support the hypothesis that the PlA2 polymorphism represents an added inherited platelet risk factor for migraine or even migraine with aura, further searches for such a factor are clearly warranted, because of the familial aggregation of migraine headache cases.

Thus this preliminary study shows that PlA1/A2 polymorphic state on the GPIIIa platelet membrane receptor does not increase the risk of inheriting migraine. However if present, it is more likely to manifest as migraine with aura in the migraineurs with this polymorphism.

INTRODUCTION

1.0 Introduction

1.1 Migraine: a complex disease

Migraine is a common disabling disorder characterized by the recurrence of painful and at times non-painful episodic phenomena associated with a variety of neurological manifestations. It ranks among the top 20 causes of disability worldwide (Stewart WF et al., 1999).

Migraine may be considered as a chronic illness (migraine seen as a 'disease') interspersed with acute signs and symptoms (migraine seen as an 'attack'). Migraine is characterized by a great variability of phenotypic expressions.

The clinical heterogeneity of migraine involves both the attacks and the disease. Another important aspect of the heterogeneity is the significant association between migraine and other neurological diseases (epilepsy, episodic ataxia, cerebrovascular disorders, mitochondrial diseases), psychiatric illnesses (anxiety, mood and personality disorders), and cardiovascular disorders (arterial hypertension, mitral valve prolapse) (Nappi G et al., 2000). The co-morbidity of migraine, may be the result of genetic factors such as the presence of different mutations in the same gene (allelic disease) or mutations in genes located on neighbouring segments of the same chromosome (Ducros A et al., 2002)

1.2 Prevalence and frequency of migraine

Migraine is a highly prevalent headache disorder that has a substantial impact on individuals and on society. Migraine is now ranked by the World Health Organization as the number 19th among all diseases causing disability (Jes Oleson et al., 2003). One-year migraine prevalence in the general population for Western countries varies from 4% to 9% in men and 11% to 25% in women (Lipton RB et al., 2001).

A community based study on headaches in Malaysia showed the prevalence of migraine to be 9 % in the population (Alders EE et al., 1996). Migraine has been found to be two to three times more prevalent in women than in men (Lipton RB et al., 2001). Migraine prevalence has also been found to be age-dependant. In children, the prevalence of migraine increases with age, with male preponderance before puberty and female preponderance thereafter (Lipton RB et al., 2001). In adults, the prevalence of migraine in women increases with age peaking at the fourth and fifth decade after which it declines. A similar trend is seen with males. The effect of gender on migraine prevalence may be only partially explained by hormonal factors because gender difference persists after the menopause (Pascual J et al., 2001).

Migraine has been shown to be common in all races but significantly higher in Caucasians, when migraine prevalence has been studied in African Americans or Asian Americans. These data suggest that race- related differences cannot be explained only by cultural and environmental factors and that a different genetic vulnerability may be

important. Several lines of evidence supports that genetic factors are involved in migraine, especially in migraine with aura compared with migraine without aura (Russel MB and Olesen J, 1995). Studies of familial aggregation have revealed that first-degree relatives of probands who had migraine with aura had a four-fold increased risk of migraine with aura. The most likely mode of inheritance is multifactorial (Russel MB and Olesen J, 1995).

1.3 Pathophysiology of migraine

The mechanisms underlying migraine have not been fully understood. Several theories have been proposed on the pathogenesis of migraine which are: the vascular theory, the cortical spreading depression theory, the neurovascular hypothesis and the brainstem "migraine generator" theory.

1.3.1 The Vascular Theory

The vascular theory, as proposed in 1938 by Graham and Wolff attributes migraine to an initial intra-cranial arterial vasoconstriction, resulting in ischemia to the visual cortex and symptoms of aura, followed by a period of extra-cranial vasodilatation resulting in the throbbing headaches (Wolff HG, 1938). It has been pointed out by subsequent workers time and again, that the entire syndrome of migraine cannot be explained merely on the basis of vascular constriction and dilatation only.

1.3.2 The Cortical Spreading Depression Theory

This neuronal theory of Cortical Spreading Depression (CSD) observed initially by Leao (Leao AAP, 1944) envisages an electrical phenomenon. CSD is a relatively short-lasting wave of depolarization that originates from the cerebral cortex and spreads across the surface of the brain, usually moving from the back (occipital region) of the ipsilateral cerebral cortex toward the front and laterally at about 3-6 mm/minute (Lauritzen M, 1994). According to the theory, CSD begins with a brief wave of excitation, followed by a prolonged period of neuronal depression, which is associated with disturbances in nerve cell metabolism and regional reductions in blood flow. This disturbance spreading along the visual cortex causes the initial flickering phenomenon during the irritative phase and then leads on to the scotoma representing the depressed neuronal function phase. Similarly, sensory aura in the form of tingling on the surface of the body has also been observed in some cases of migraine. Clinical observations, neuro-imaging techniques and blood flow measurements clearly indicate CSD to be the primary underlying factor for the aura and not cerebral ischemia as was previously thought (Cutrer FM et al., 1998).

1.3.3 The Neurovascular Hypothesis

The neuro-vascular theory implicates the trigemino-vascular neural network as the major pain-signalling system, wherein the perivascular trigeminal axon terminals release antidromically certain neuropeptides (including substance P, neurokinin A, calcitonin gene-related peptide (CGRP and others) during the migraine attacks resulting in neurogenic inflammation causing vasodilatation, mast cell degranulation,

increased platelet aggregation and alterations in the vascular permeability. This neurogenic inflammation leading to the central transmission of pain impulses is believed to cause the migraine headache phenomenon.

1.3.4 The Brain stem "migraine generator" theory

The dorsal raphe nucleus in the peri-aqueductal gray matter in the brainstem has high concentration of serotonin which happens to be an important central neurotransmitter. This nucleus also contains serotonin-secreting neurons whose axons terminate on cerebral blood vessels and various other brain areas that are involved in the production of migraine symptoms. It has thus been suggested that the raphe nucleus, which is sensitive to the changes in blood serotonin levels, may serve as a "migraine generator" (Curran DA et al., 1965) leading on to the cascading of the migraine syndrome. The demonstration of persistent brainstem activation during and after an attack of migraine as imaged in Positron emission topography (PET) scans lends support to this theory (Cutrer FM et al., 1998).

1.3.5 Current Concept of Migraine – Genetic basis

All the four theories listed above have their own merits and serve to explain some, if not the entire migraine syndrome. Migraine being a common problem, with many people in the world being disabled with headache, enormous literature on the understanding and treatment of the condition continues to be accumulated.

Genetic epidemiology has shown that the risk to first-degree relatives of probands with migraine with aura is fourfold, whereas in migraine without aura it is only 1.9 fold, clearly indicating that genetic influence is stronger in migraine with aura (Russell MB et al., 1996). However no clear cut mutation on any particular chromosome has been detected. On the contrary, familial hemiplegic migraine patients have been shown to have abnormal mutations in the brain-specific P/Q type calcium channel alpha 1A subunit gene (CACNA1A) on chromosome 19p13 (Joutel A et al., 1993). Abnormal mutations are now being postulated which can open up newer therapeutic strategies in the prophylaxis and treatment of migraine. Thus the role of genetic predisposition to migraine is becoming more and more established and this seems to be an important prerequisite in the genesis of migraine. It stands to reason therefore that genetic work undertaken to study the DNA of the migraine patients with aura is more likely to yield interesting results than in those without the aura.

The simplest way to explain these peculiarities of migraine is to consider the basic mechanism as a genetic abnormality resulting in a lowered response threshold of the central nervous system (CNS) to specific trigger factors determining the initiation of migraine attack. In this model, the trigger factors are considered as modulators of genetic set-point that predisposes the individual to migraine. This inability to handle migraine trigger factors results in a primary CNS dysfunction that secondarily activates headache pain. The sequence of events can be schematically represented as shown in Figure1.

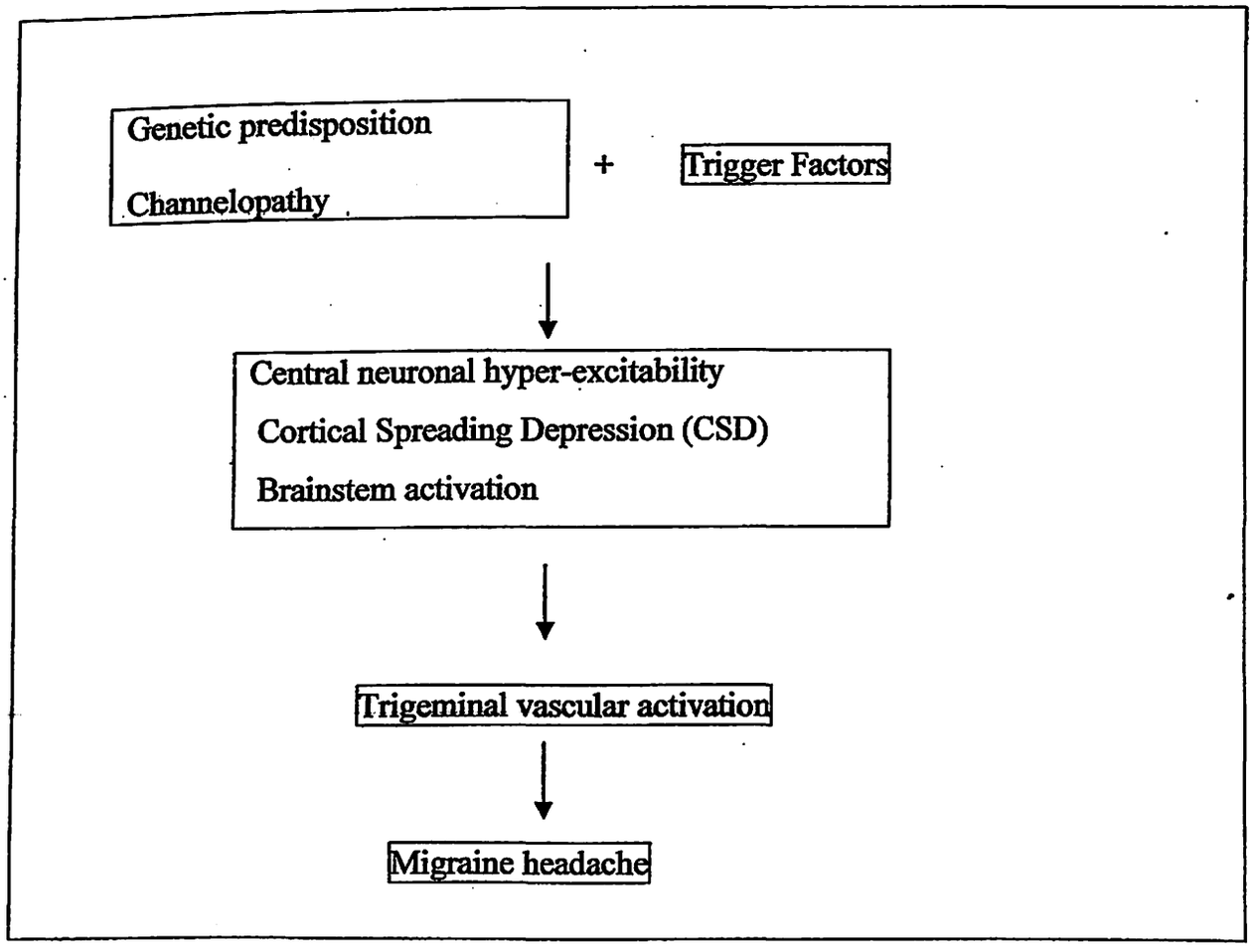

Figure 1 Genetic predisposition to migraine

Adapted from Handbook of Headache by Randolph W. Evans 2005

It appears that Cortical Spreading Depression (CSD) is the first instrumentally recordable phenomenon in the migraine cascade. The CSD probably gets triggered by many extraneous factors including hormonal triggers (menstruation, ovulation and pregnancy), dietary triggers (alcohol, chocolates,cheese), psychological triggers, environmental triggers (glare, obnoxious odours), lack of sleep and certain drugs (Oestrogens, reserpine, nitroglycerine).

The triggered CSD leads to excitation of the trigeminal vascular afferents directly which is followed by vasodilatation of the dural and pial blood vessels and the large blood vessels of the brain. The dilated blood vessels in turn stimulate the nerve endings which release neuropeptides such as calcitonin-gene related peptide (CGRP), substance-P and neurokinin-A. These further dilate the blood vessels and cause a local inflammatory reaction (plasma extravasation and mast cell degradation) that transduce pain at the trigeminal nerve endings (first order neurons). Activation of the trigeminal vascular system is the primary mechanism of pain in migraine (Evans RW and Mathew NT, 2005).

Activated first order trigeminal neurons then transmit pain impulses to the second order neurons in the trigeminal nuclei which interconnects with the various other brainstem nuclei including the nucleus solitarius resulting in nausea and vomiting. (Goadsby PJ, 1997).

The second order trigeminal neurons transmit the pain impulses rostrally to the thalamus where the third order neurons take over and convey the nociceptive and other impulses to the cortex, ultimately resulting in the other later symptoms like photophobia, phonophobia, osmophobia, allodynia, impaired cognition and attention and hyperirritability.

The upper brainstem nuclei, apart from housing the migraine generators, have yet another role to play in modulating the upward transmission of the pain impulses by decreasing the firing rate of the serotonergic cells.

1.4 Platelets, Serotonin and Migraine

Observations that both plasma and platelet levels of serotonin fluctuate during a migraine attack suggest that serotonin may be involved in the pathogenesis of migraine. When platelets are activated, they aggregate and release serotonin, thus increasing the plasma serotonin level. This increase in plasma serotonin level leads on to initial vasoconstriction; and the subsequent depletion and abrupt fall in the serum serotonin level results in intense rebound vasodilatation manifested clinically as the throbbing headaches. Platelet serotonin levels have been observed to drop precipitously during the headache phase of migraine. Also, the urine levels of serotonin and its metabolites rise suggesting that there had been a large release of serotonin into the plasma during such attacks. The changes in plasma serotonin levels also reflect the more important disturbances in brain serotonin levels.

The tendency of platelets to get easily activated leading to hyperaggregation has been adequately documented in migraineurs (Couch J.R and R.S., 1976). The platelets of migraine patients are more sensitive than in normal individuals in their response to several vasoactive amines, including serotonin, catecholamine and tyramine (Kovac K et al., 1990).

Secondly, platelets contain over 90 percent of the serotonin (5-hydroxytryptamine, 5-HT) in the blood. At the onset of an attack of migraine a significant rise in the plasma concentration of serotonin has been observed (Dvilansky A et al., 1976). Even during the headache-free periods, migraine patients were shown to exhibit greater degrees of platelet sensitivity to serotonin and adenosine diphosphate than healthy controls (Lechner H et al., 1985). The work of Sicuteri and associates and Curran and colleagues also confirmed the role of serotonin and its metabolites during all phases of a migraine attack. These investigations demonstrated that serotonin-releasing agents can induce migraine-like attacks. The fact that many of the newer drugs used for management of acute migraine are serotonin receptor agonists further highlights the fact that serotonin is a key player in migraine pathogenesis. The various serotonergic mechanisms of migraine are summarized in Figure 2.

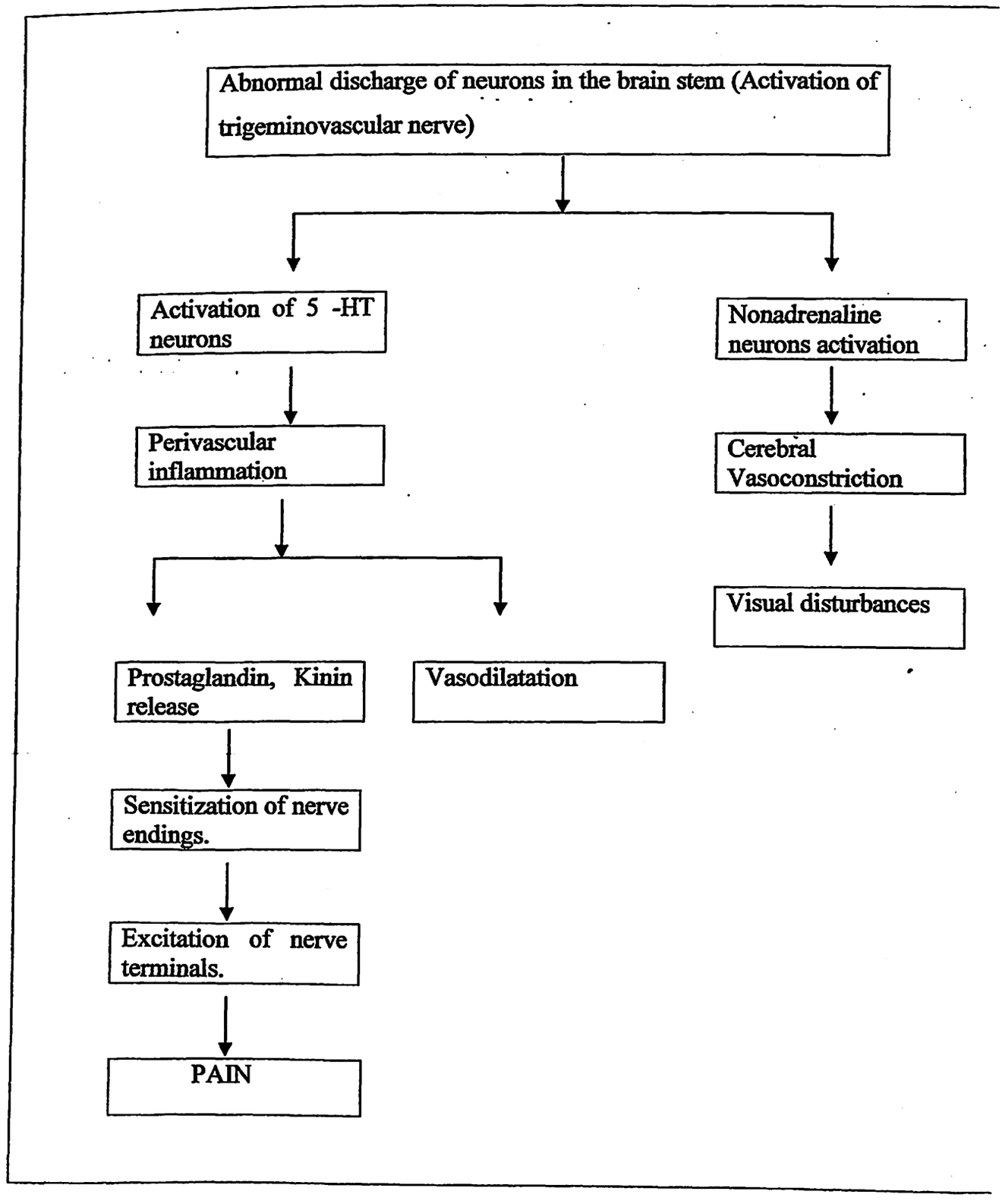

Figure 2 Serotonergic mechanisms of migraine in the CNS

Adapted from Migraine Headache - An Update by H. S. Mahajan et al 2004

5HT is a transmitter in the central nervous system and present in high concentration in localized region of the midbrain. Certain events or substances can set off an imbalance of naturally occurring chemicals in the brain, causing the blood vessels of the head to expand. The area around these blood vessels becomes inflammed and irritates nerve endings. This dilation and irritation may account for the throbbing pain.

It must finally be pointed out that though much has been written on platelets, serotonin and migraine, the exact inter-relationship between the following key observations in cases of migraine has not been fully worked out. They may well be independent of one another and not causally related (i.e) platelet dysfunction of hyperaggregability and increased sensitivity to vaso-active amines in patients with migraine and activation of the serotonergic neurons at the brainstem level during attacks.

Still, the co-existence of the above factors noticed in cases of migraine points out to some hitherto unexplored association.

1.5 Physiology of platelet function

1.5.1 Platelet Adhesion, Aggregation and Thrombus formation

Platelet activation leads to adhesion and can be initiated by several mechanical and chemical stimuli. Platelet aggregation with subsequent thrombus formation is the key event in the development of acute coronary syndromes and sudden death of patients with coronary heart disease (Chesebro JH., 1992).

Platelet adhesion is triggered by damage to the vessel wall and local exposure to the subendothelial matrix. Coverage of the exposed site by platelets depends on the recognition of adhesive proteins by specific platelet-membrane glycoproteins, many of which are intergrins (Hynes RO, 1987, Smyth SS et al., 1993).

Integrins are found on virtually all cells and they mediate many different physiologic responses. The major integrins present on the surface of platelets are listed in Table 1 and all of them have been shown to play a part in the process of platelet adhesion.

Several receptors, each with distinct functions are involved in the process of thrombus formation. These include glycoprotein (GP) Ib-IX-V complex, the integrins $\alpha_2\beta_1$ and $\alpha_5\beta_1$ (Savage B et al., 1998) and platelet GP VI. As a result of these initial interactions, platelet is activated, leading to a change in another integrin, $\alpha_{IIb}\beta_3$ which contributes to the homotypic aggregation of platelet into a thrombus. The first receptor to be scrutinized was the integrin $\alpha_{IIb}\beta_3$ (initially designated glycoprotein IIb-IIIa), the most abundant receptor on the platelet membrane surface.

The rate of platelet activation is the important variable that contributes to either beneficial or adverse outcomes. In view of their key role in thrombosis, it is natural to wonder whether polymorphisms in platelet receptors can influence the risk of cardiovascular disease. In the last few years, there has been a rapid accumulation of literature concerning the relationship between genetic variations in platelet glycoproteins and risk for coronary artery disease. Since the initial report by Weiss et al in 1996, that PlA2 polymorphism is a cardiovascular risk factor, no fewer than 17

studies have presented findings on this topic. Of these, nine support a role for the PlA2 allele as a thrombotic risk factor, and eight do not (Tatiana V et al., 2000).

The underlying platelet hyperaggregability and vascular involvement as observed in the pathophysiology of migraine suggests the plausibility of an association between migraine and coronary heart disease (CHD). However the literature is inconclusive on this subject. Population-based studies report both an increased occurrence of CHD among migraineurs (Mitchell P et al., 1998) as well no association between the two illnesses (Cook NR et al., 2002).

Table 1 **Platelet-Membrane Glycoprotein Receptors involved in the Adhesion and Aggregation of Platelets.**

Receptor	Ligand	Receptor– Mediated Action
Integrin		
$\alpha_2\beta_1$(glycoprotein Ia/IIa)	Collagen	Adhesion
$\alpha_5\beta_1$ (glycoprotein Ic/IIa)	Fibronectin	Adhesion
$\alpha_6\beta_1$	Laminin	Adhesion
$\alpha_{IIb}\beta_3$ (glycoprotein IIb/IIIa)	Fibrinogen Fibronectin von Willebrand factor Vitronectin	Aggregation
$\alpha_v\beta_3$	Vitronectin Fibrinogen Fibronectin von Willebrand factor	Adhesion
Nonintegrin		
Glycoprotein Ib	von Willebrand factor Thrombospondin	Adhesion Adhesion
Glycoprotein IV	Collagen	

Adapted from Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine

Jeffrey Lefkovits et al 1995

1.5.2 Structure and Function of the GP IIb-IIIa Receptor

The human platelet membrane glycoprotein (GP) IIb-IIIa complex (integrin $\alpha_{IIb}\beta_3$) mediates platelet aggregation via the binding of adhesive proteins, such as fibrinogen and von Willebrand factor (vWF) (Phillips et al., 1988).

The GPIIb/IIIa receptor consists of a 2-chain glycoprotein IIb subunit noncovalently associated with a single-chain glycoprotein IIIa subunit (Figure 3). Its 136-kd α subunit consists of a heavy chain and a light chain. The 92-kd β subunit consists of a single polypeptide of 762 amino acids. Of the eight β integrin subunits, the aIIb subunit has been found only in combination with β3, mostly in cells of the megakaryocyte lineage. The subunits are noncovalently bound to each other, and to fibrinogen, von Willebrand factor, fibronectin, vitronectin, and a number of other adhesive proteins ~~resulting in heterodimers~~ (Lefkovits et al., 1995).

The genes encoding glycoprotein IIb and IIIa are located on chromosome 17q21 (Thornton MA et al., 1999). Integrins are receptor proteins that promote interactions between cells and their environment and they also act as cellular sensors and signalling molecules. Integrins are found virtually on all cells and mediate many different physiologic responses.

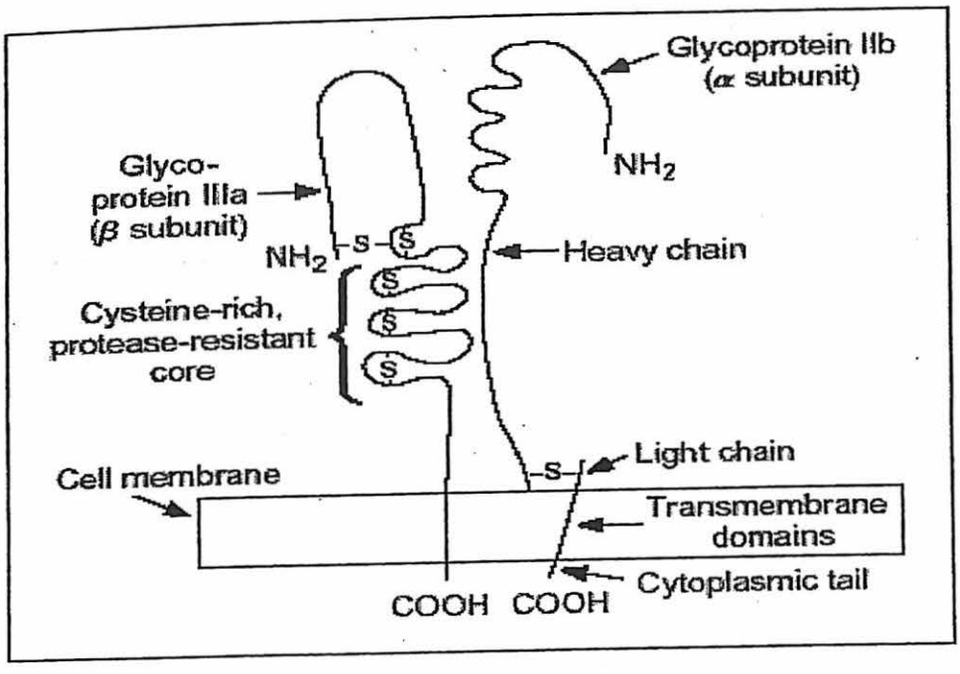

Figure 3 Diagram of Platelet Glycoprotein IIb/IIIa Receptor

NH_2 denotes the amino group, COOH the carboxyl group, and S the disulfide group.

Adapted from Plow et al 1992

Three allelic variants of the integrin subunit αIIb have been defined which differ at either residue 837 (Val or Met) (Noris P et al., 1995) or residue 843 (Ser or Ile) (Lyman S et al., 1990). Eight different alleles of the β3 can be distinguished which differ at six positions within the coding sequence. These dimorphic residues and their serologic designations include Leu33/Pro33 (HPA-1a/HPA-1b) (Newman PJ et al., 1989). The molecular basis of this polymorphism is a thymine-cytosine change at position 1565 in exon 2 of the GPIIIa gene (also known as integrin β3-ITGB3 gene) (Reiner A et al., 2001). Amino acid substitutions in platelet membrane glycoprotein result in alloantigens. As a result human platelet alloantigen (HPA-1a or PlA1) molecules have a leucine, whereas HPA-1b or PlA2 proteins have a proline, at position 33 of the mature β3 integrin chain (Newman PJ et al., 1989). These inherited polymorphisms within the platelet membrane glycoprotein genes can alter their antigenicity, regulate their expression levels and modulate their functional properties.

Shulman NR et al had shown that the PlA system was the first recognized platelet-specific alloantigen implicated in neonatal alloimmune thrombocytopenia (NAIT) and maternofetal incompatibility for PlA antigens accounts for more than 90 % of reported cases of NAIT as well as most cases of post transfusion purpura (Mueller-Eckhardt C et al., 1989). Recent studies done on GPIIIa gene have shown that possession of an A2 allele increases the tendency for platelet adhesion and hence the risk for myocardial infarction (Weiss EJ et al., 1996) (Anderson JL et al., 1999) coronary artery disease (Gardemann A et al., 1998) and restenosis after stent placement (Kastrati A et al., 1999)

The platelet GPIIb/IIIa receptors have an important role in thrombus formation (Lefkovits et al., 1995). Platelet activation causes changes in the shape of platelets and conformational changes in glycoprotein IIb/IIIa receptors, transforming the receptors from a ligand-unreceptive to a ligand-receptive state. Ligand-receptive glycoprotein IIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of glycoprotein IIb/IIIa receptors also bind to glycoprotein IIb/IIIa receptors, blocking the binding of fibrinogen and thus preventing platelet aggregation (Figure 4).

Several point mutations in the genes that encode GPIIb and GPIIIa result in disorders of platelet binding. As techniques to identify mutations in the genes encoding these glycoproteins have become available, interest has grown in the possibility that some of these mutations may represent independent risk factors for vascular thrombosis. The study by Weiss and colleagues had suggested that the platelet GPIIIa polymorphism PlA1/A2 is an important inherited risk factor for acute coronary ischaemia.

In their retrospective case-control study, patients with coronary thrombosis were more likely than controls to carry the PlA2 allele, especially those who were 60 years or younger at the time of infarction. Since the estimated frequency of the PlA2 allele among northern and central Europeans is 15%, (S Simsek, 1993), this inherited defect of platelet binding could contribute substantially to the pathogenesis of thromboses. This discovery has opened up novel therapeutic approaches with treatment and prevention trials using GPIIb/IIIa inhibitors; and attempts to identify high-risk subgroups are also being made (Coller BS, 1995).

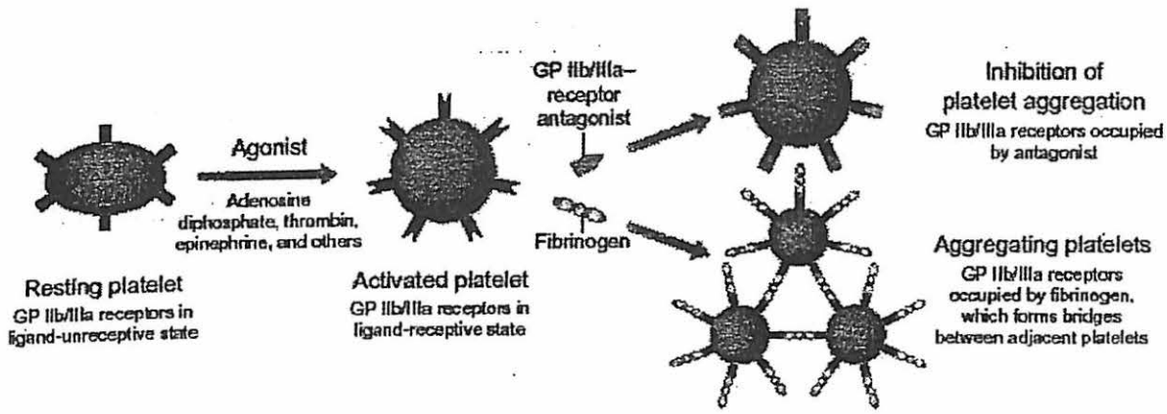

Figure 4 Overview of the Processes of Platelet Activation and Aggregation and the Inhibition of Platelet Aggregation by Inhibitors of GP IIb/IIIa Receptors.

Adapted from Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine Jeffrey Lefkovits et al 1995

1.6 Principles of Polymerase Chain Reaction (PCR)

The Polymerase Chain Reaction (PCR) in molecular study permits the selective in vitro amplification of a particular DNA region with a pair of oligonucleotide primers (Saiki R et al., 1988). As a result a million fold of products are produced. The essential components for PCR amplification are;

i) the strands of a DNA double helix run in an opposite polarity along the backbone, such that one strand is viewed as extending in the 5' to 3' direction, the other strand runs in the 3' to 5' direction;

ii) a target sequence in a DNA sample that lies between the pair of primers and can be form 100 to ~35,000 base pair in length;

iii) a thermostable DNA polymerase that can withstand heating to 95°C or higher;

iv) oligonucleotides of about 15-17 bases will normally hybridize to a unique site on a DNA.

A PCR reaction consists of a number cycles for amplifying a specific DNA sequence. Each cycle has three successive steps: denaturation, annealing and synthesis. In the first step of a PCR reaction, the DNA is denatured by heating to 93°C to 95°C so that the strands come apart. In the second step the reaction is cooled to a lower temperature, typically 50-72°C such that the primers anneal selectively to the regions which flank the target site to be amplified. Lastly, the reaction is heated to 72°C to 75°C and the DNA polymerase extends the primers

such that a copy of the target region is synthesized. Typically, this three steps sequence is repeated for 25 to 35 cycles.

The elegant technique of PCR, by which fragments of DNA can be made to replicate very rapidly, is illustrated in Figure 5.

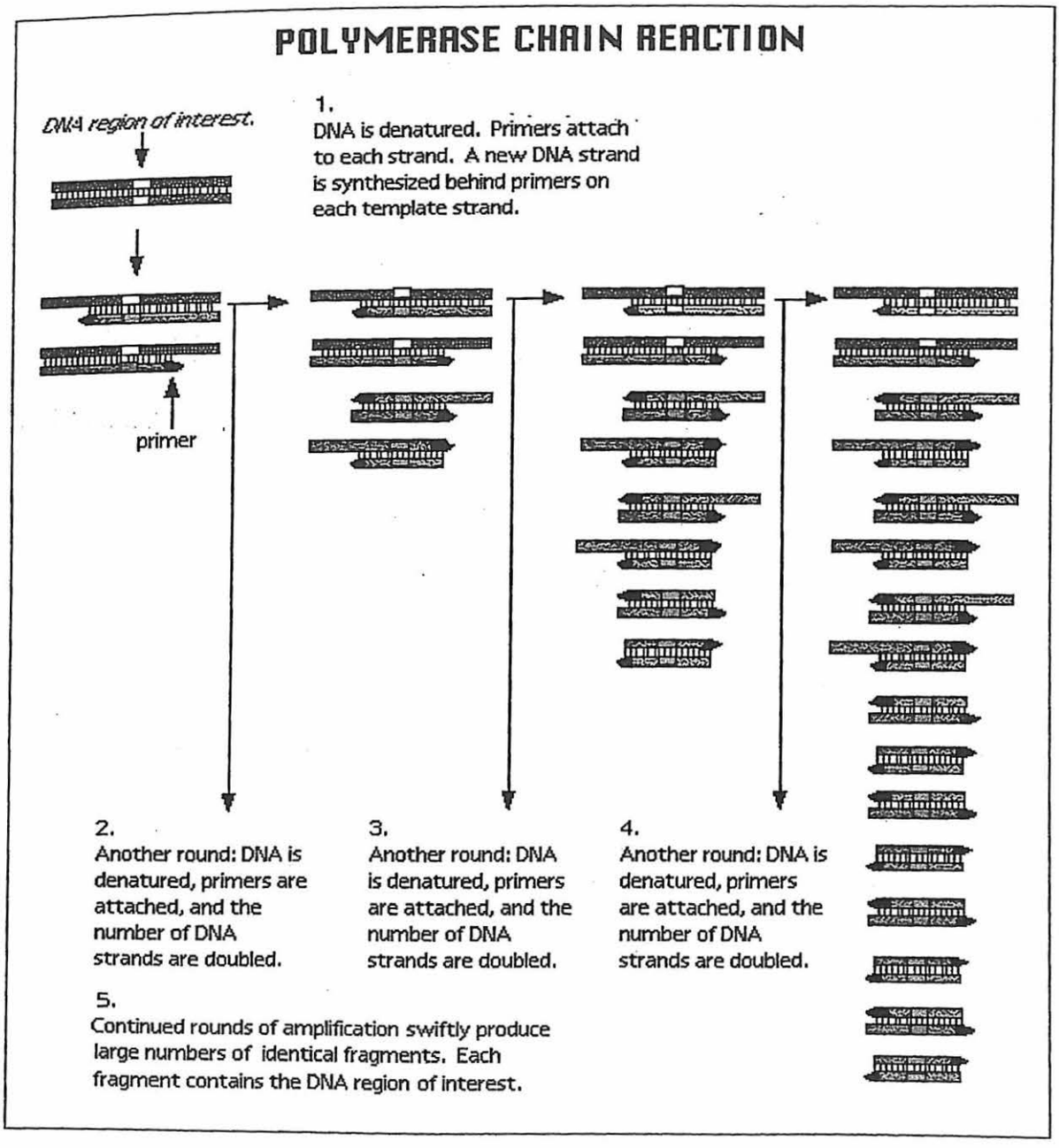

Figure 5 The PCR cycle sequence

Adapted from Access Excellence @ the National Health Museum Copyright 1999

Commonly, oligonucleotides of about 20 nucleotides long are used as primers. Primers should not contain a sequence with any internal secondary structures, especially at the 3' end because the oligonucleotide may not anneal properly. In addition the primers should not be able to hybridize to each other in order to avoid the synthesis of primer dimers. With any primer it is desirable to confirm that the sequence is not found in other parts of the genome (Saiki R et al., 1988). The G C content of the primer will affect the reaction temperature. It is therefore desirable to make the G C contents of the two primers similar.

The reaction condition for each experiment may vary and has to be optimized to obtain the best results. The annealing temperature must be low enough to get sufficient annealing to start the PCR reaction, but high enough to prevent nonspecific priming. The optimum reaction conditions often differ for different DNA polymerases and different PCR machines (Innis MA et al., 1990).

1.6.1 Methods for detection of PlA1/PlA2 genotypes

Detection of PlA1/A2 polymorhism can be carried out by various methods. Two main approaches have been used for platelet genotyping, both based on PCR technique: allele specific oligonucleotide (ASO) hybridization (McFarland JG et al., 1991) and digestion PCR-amplified material with restriction endonucleases (restriction fragment length polymorphism; RFLP) (Newman PJ et al., 1989).